HSB Project 8

Impact of Sex and Strain on the Performance of Genomic Signatures of Hepatocarcinogenesis

Project Leader
Scott S. Auerbach, Ph.D.

Background and Rationale
Only a small fraction of the chemicals currently in commerce have undergone carcinogenicity testing (Judson et al., 2009). Due to the combination of a broad landscape of untested chemicals and the current limitations of the bioassay there has been no shortage of attempts to identify methods that will allow for more rapid identification of potential human carcinogens. One approach that has taken on some favor in the toxicology community involves the application of genomic technology in combination with machine learning (Kramer *et al.*, 2004; Nie *et al.*, 2006; Fielden *et al.*, 2007; Thomas *et al.*, 2007; Ellinger-Ziegelbauer *et al.*, 2008; Uehara *et al.*, 2008). Most of these studies have focused on liver because it is a common target for chemical-induce carcinogenic transformation.

Recently, the NTP successfully applied a genomics-based approach to estimate the hepatocarcinogenic hazard associated with a number of alkenylbenzene flavoring agents (Auerbach *et al.*, Submitted). Although there has been some effort to validate a select number of predictive signatures across laboratories (Fielden *et al.*, 2008), there has been limited consideration of a number of additional variables that are likely to influence signature performance, such as sex and strain of the animal used to derive training and test data. Here we propose genomics-based studies that employ male and female F344/N, Harlan Sprague Dawley (HSD) and Wistar Han rats that are specifically designed to address the performance of signature-based classification across strain and sex. In short, male F334/N rats will be exposed to a structurally diverse collection of approximately 30 chemicals (this set of chemicals will include genotoxic hepatocarcinogens, non-genotoxic hepatocarcinogens, hepatotoxic non-carcinogens and non-toxic non-carcinogens). Genomic expression changes elicited by the chemical treatments will be used to train a machine learning model. The model will be used to classify gene expression changes elicited in female F344/N and male and female HSD and WH rats by treatment with a subset of 15 chemicals (10 from the training set and 5 not used to train the models).

Key Issues
F344/N, Sprague Dawley and WH are three of the most commonly used strains for carcinogenicity testing. Up to 50% of the genetic loci in these strains are divergent (Kloting *et al.*, 2003). Therefore, use of these strains will make the studies immediately relevant to the toxicological testing community and should adequately address the variable of genetic diversity.

Chemical and dose selection for these studies will be a critical determinant of success in particular, because carcinogenic potency may vary considerably between strains and in most cases, 2-year bioassays have been performed in only one strain of rat. To provide greater certainty as to the carcinogenic effects across the three strains and sexes, selection preference will be given to chemicals that have been evaluated for carcinogenicity in two or more strains of rat and those that have been tested in both sexes. Where there are clear differences in potency between strains or sexes, doses will be adjusted appropriately. To add further certainty that the carcinogens employed in these studies would have a carcinogenic effect in strains not used in the 2-year bioassay, they will be administered at a dosed level that is at or above the TD50 (dose anticipated to produce tumors in 50% of the animals after two years of exposure). To increase the likelihood that the dose of the non-carcinogens would be non-hepatocarcinogenic in the three strains and both sexes, selection preference will be given to those chemicals that were negative for carcinogenicity in two sexes and two species.

Exposure duration can determine the qualitative and quantitative changes elicited by chemical treatment. In order to obtain changes in gene expression essential for creating robust predictive models, it is possible to administer doses for short durations (1 to 14 days) which exceed a 90-day MTD; however, such an approach has the potential of introducing confounding effects on gene expression that are not relevant to dose levels that would be used in a carcinogenicity bioassay. Doses at the 90-day MTD or lower do often do not produce robust changes in gene expression after 14 days of exposure. Considering that we propose using doses that approximate those that would be used in a 2-year study (less than or equal to the MTD) and the desire to create models with strong biological relevance to the bioassay, we propose all chemical exposures be carried out for 90 days.

Approach and Specific Aims

1. Create a 4-class hepatocarcinogenicity prediction model (4 classes: genotoxic hepatocarcinogen, non-genotoxic hepatocarcinogen, hepatotoxic non-carcinogen, and non-toxic non-carcinogen) trained on hepatic gene expression from male F344/N rats exposed to 30 distinct chemical treatments.
2. Determine the classification accuracy of the model when it is used to classify gene expression from a distinct sex and strain of rat.

Significance and Expected Outcome

These studies will identify a robust, multi-class model that predicts carcinogenic activity at one of the most common sites of carcinogenic action in rodents. In addition, it will determine the degree to which a genomic signature of carcinogenic activity derived from one sex/strain can be used to predict carcinogenic activity in a distinct sex/strain. These data should decrease the uncertainties associated with the application of genomic signatures for decision making in toxicology and risk assessment.

Current Activities

Chemical and dose selection are under way and initial studies should be started this fiscal year.

Future Plans

We plan to use a similar approach to determine if predictive signatures can accurately classify gene expression changes in other species (mouse).

References

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